```
EcoRIa                                24                                39                                54
GAATTCCGG TGC AGG ACG AAG CTG TTC TGG ATT TCT TAC AGT GAT GGG GAC CAG
                Arg Thr Lys Leu Phe Trp Ile Ser Tyr Ser Asp Gly Asp Gln

                        69                                84                                99
TGT GCC TCA AGT CCA TGC CAG AAT GGG GGC TCC TGC AAG GAC CAG CTC CAG TCC
Cys Ala Ser Ser Pro Cys Gln Asn Gly Gly Ser Cys Lys Asp Gln Leu Gln Ser

    114                               129                               144                               159
TAT ATC TGC TTC TGC CTC CCT GCC TTC GAG GGC CGG AAC TGT GAG ACG CAC AAG
Tyr Ile Cys Phe Cys Leu Pro Ala Phe Glu Gly Arg Asn Cys Glu Thr His Lys

            174                               189                               204          Pst Ia
GAT GAC CAG CTG ATC TGT GTG AAC GAG AAC GGC GGC TGT GAG CAG TAC TGC AGT
Asp Asp Gln Leu Ile Cys Val Asn Glu Asn Gly Gly Cys Glu Gln Tyr Cys Ser

219                               234                               249                               264
GAC CAC ACG GGC ACC AAG CGC TCC TGT CGG TGC CAC GAG GGG TAC TCT CTG CTG
Asp His Thr Gly Thr Lys Arg Ser Cys Arg Cys His Glu Gly Tyr Ser Leu Leu

        279                               294                               309                               324
GCA GAC GGG GTG TCC TGC ACA CCC ACA GTT GAA TAT CCA TGT GGA AAA ATA CCT
Ala Asp Gly Val Ser Cys Thr Pro Thr Val Glu Tyr Pro Cys Gly Lys Ile Pro

    Xba I       339                               354                               369
ATT CTA GAA AAA AGA AAT GCC AGC AAA CCC CAA GGC CGA ATT GTG GGG GGC AAG
Ile Leu Glu Lys Arg Asn Ala Ser Lys Pro Gln Gly Arg Ile Val Gly Gly Lys

    384                               399                               414                               429
GTG TGC CCC AAA GGG GAG TGT CCA TGG CAG GTC CTG TTG TTG GTG AAT GGA GCT
Val Cys Pro Lys Gly Glu Cys Pro Trp Gln Val Leu Leu Leu Val Asn Gly Ala

            444                               459                               474
CAG TTG TGT GGG GGG ACC CTG ATC AAC ACC ATC TGG GTG GTC TCC GCG GCC CAC
Gln Leu Cys Gly Gly Thr Leu Ile Asn Thr Ile Trp Val Val Ser Ala Ala His

489                               504                               519                               534
TGT TTC GAC AAA ATC AAG AAC TGG AGG AAC CTG ATC GCG GTG CTG GGC GAG CAC
Cys Phe Asp Lys Ile Lys Asn Trp Arg Asn Leu Ile Ala Val Leu Gly Glu His

        549                               564                               579                               594
GAC CTC AGC GAG CAC GAC GGG GAT GAG CAG AGC CGG CGG GTG GCG CAG GTC ATC
Asp Leu Ser Glu His Asp Gly Asp Glu Gln Ser Arg Arg Val Ala Gln Val Ile

                609     Sma I     624                               639
ATC CCC AGC ACG TAC GTC CCG GGC ACC ACC AAC CAC GAC ATC GCG CTG CTC CGC
Ile Pro Ser Thr Tyr Val Pro Gly Thr Thr Asn His Asp Ile Ala Leu Leu Arg

    654                               669                               684                               699
CTG CAC CAG CCC GTG GTC CTC ACT GAC CAT GTG GTG CCC CTC TGC CTG CCC GAA
Leu His Gln Pro Val Val Leu Thr Asp His Val Val Pro Leu Cys Leu Pro Glu
```

FIG. 1A

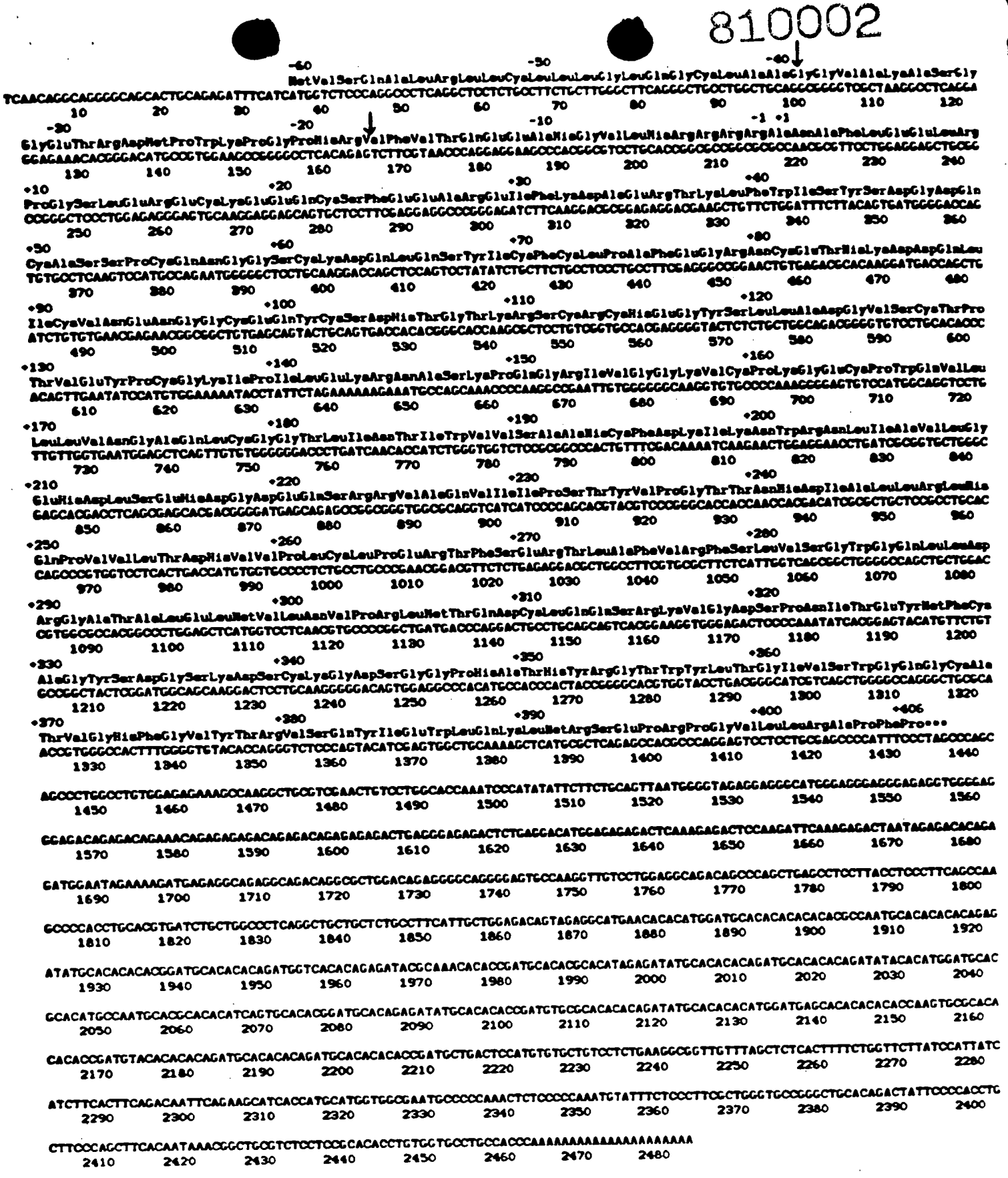
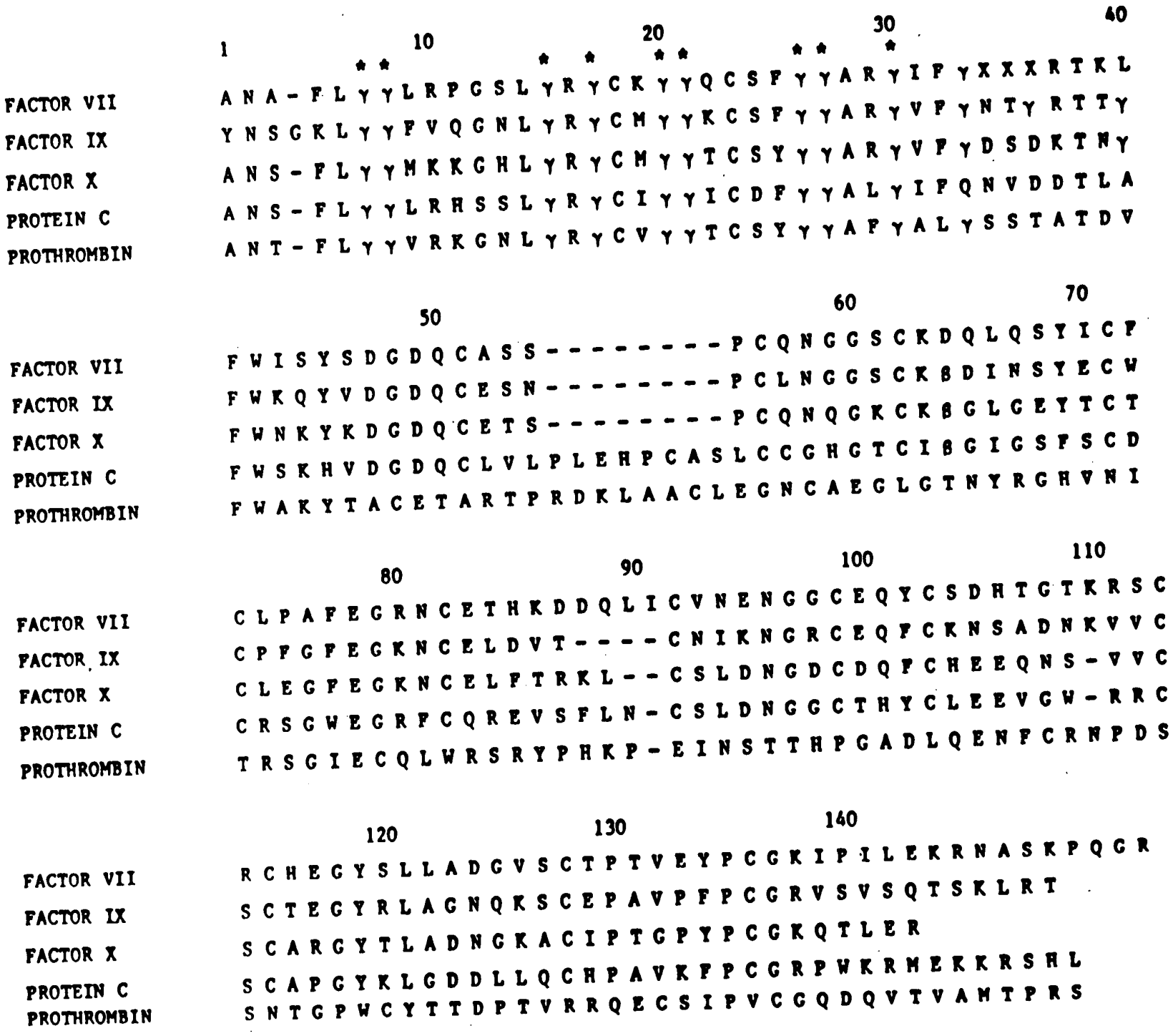


FIG. 1B

FIG. 2A



810002

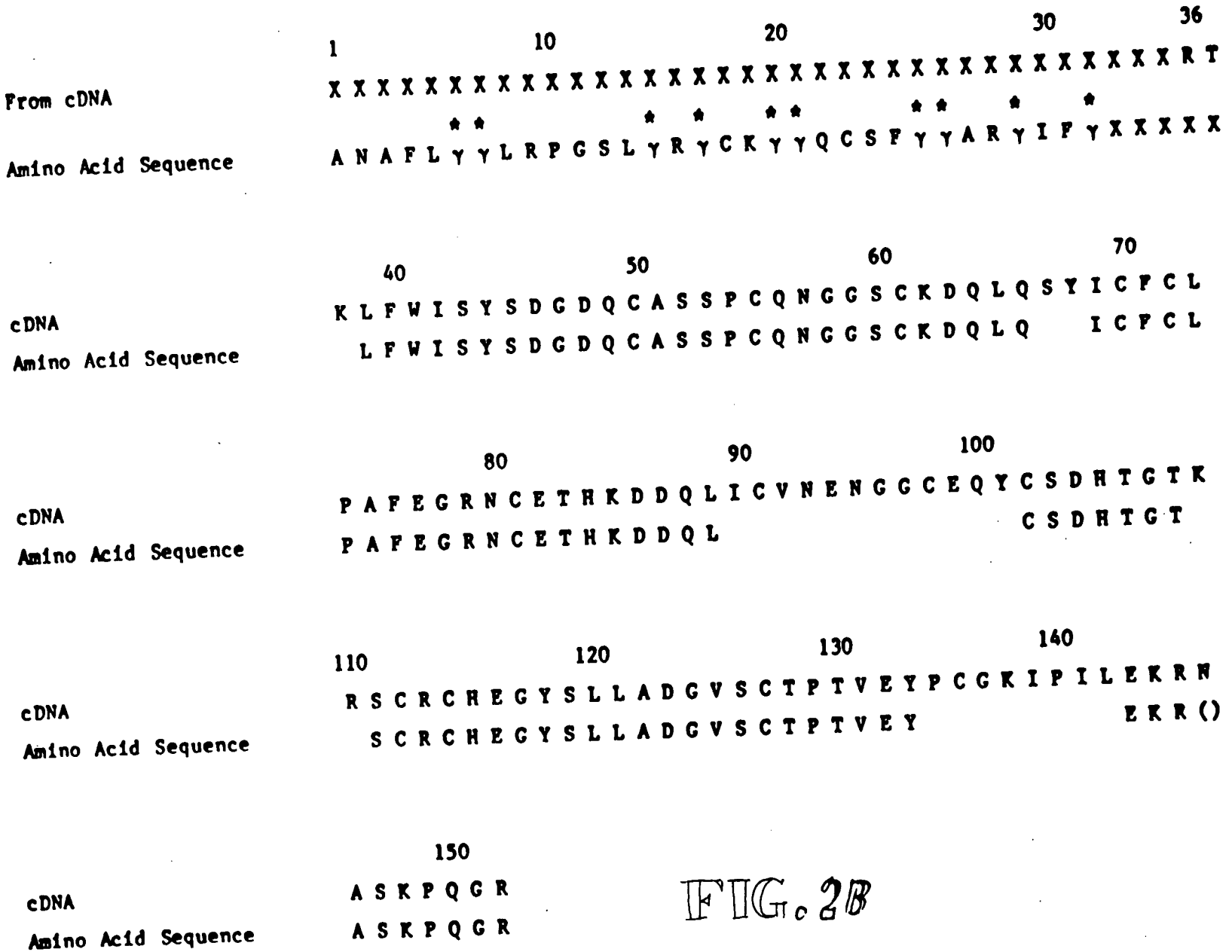
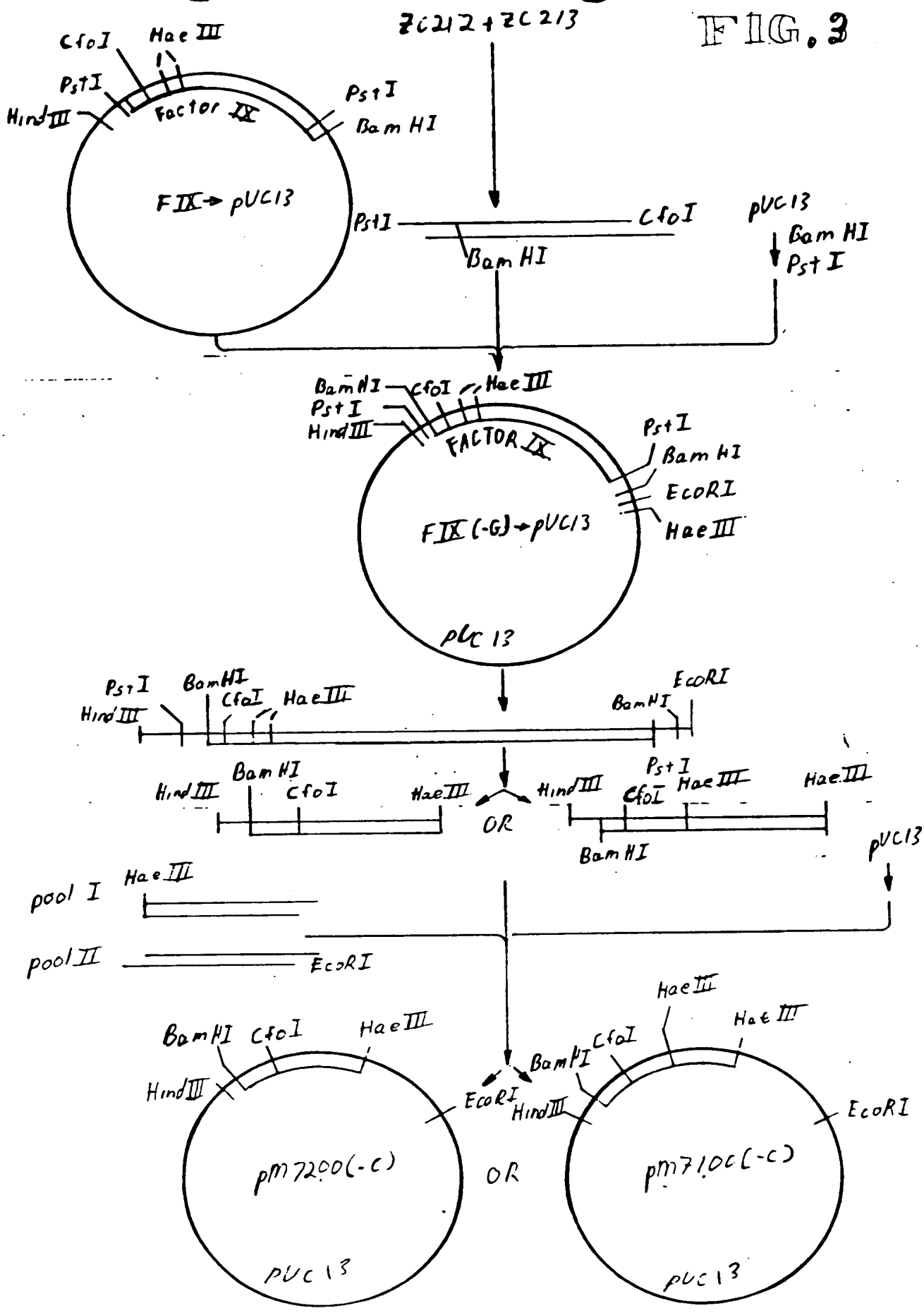


FIG. 2B

810002

FIG. 3


ZC212+ZC213
CfoI
Hae III
PstI
HindIII
Factor IX
PstI
BamHI
FIX→pUC13
PstI
CfoI
BamHI
pUC13
BamHI
PstI
BamHI
CfoI
Hae III
PstI
HindIII
FACTOR IX
PstI
BamHI
EcoRI
Hae III
FIX(-G)→pUC13
pUC13
PstI
BamHI
CfoI
Hae III
HindIII
EcoRI
BamHI
HindIII
BamHI
CfoI
Hae III
OR
HindIII
PstI
CfoI
Hae III
Hae III
BamHI
pUC13
pool I
Hae III
pool II
EcoRI
BamHI
CfoI
Hae III
HindIII
EcoRI
pM7200(-C)
pUC13
OR
Hae III
Hae III
BamHI
CfoI
HindIII
EcoRI
pM7100(-C)
pUC13

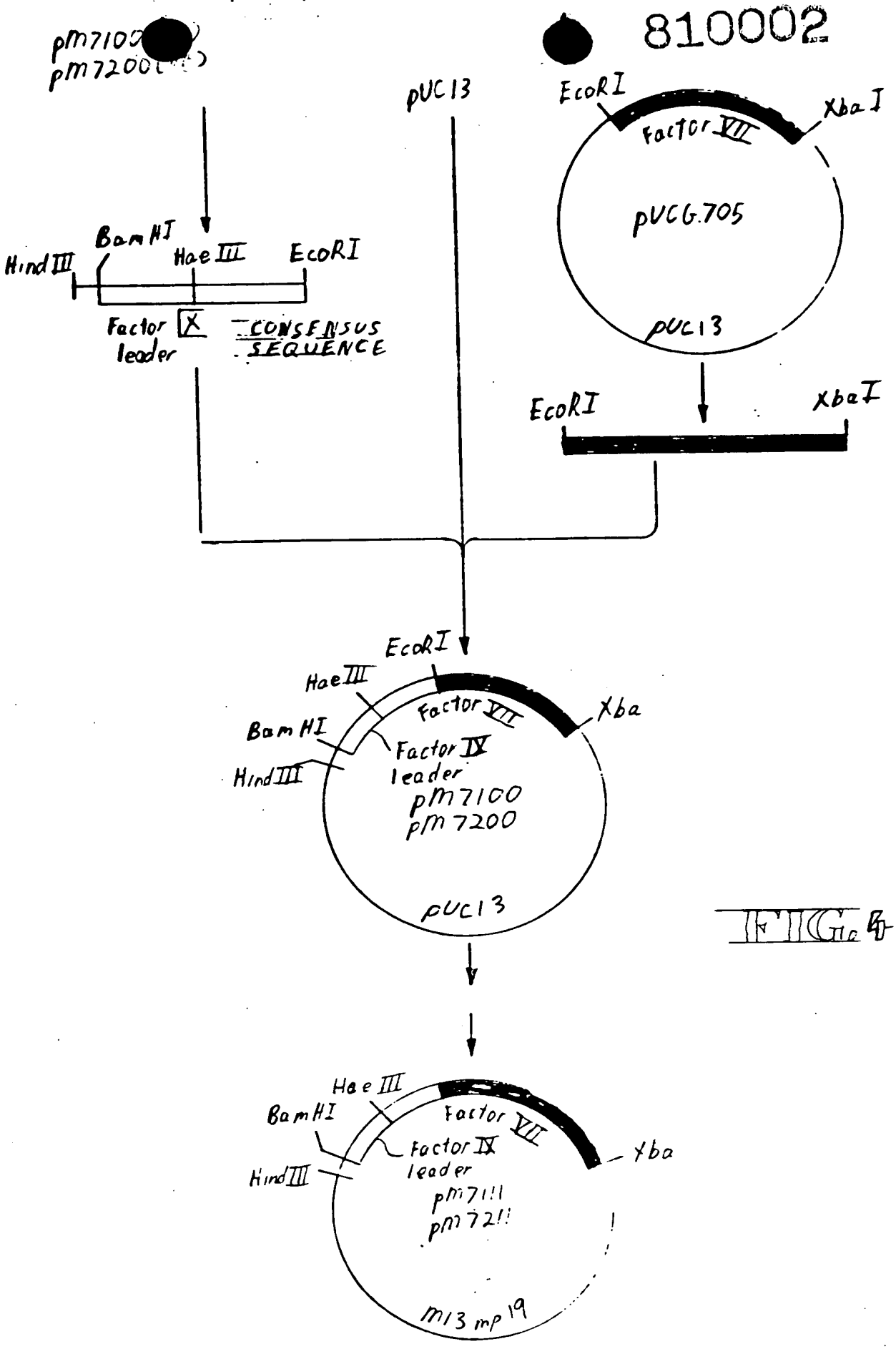

pM7100
pM7200
Hind III
Bam HI
Hae III
EcoRI
Factor IX leader
CONSENSUS SEQUENCE
pUC13
EcoRI
Xba I
Factor VII
pUC6.705
pUC13
EcoRI
Xba I
EcoRI
Hae III
Bam HI
Hind III
Factor VII
Xba
Factor IX leader
pM7100
pM7200
pUC13
Hae III
Bam HI
Hind III
Factor VII
Xba
Factor IX leader
pM7111
pM7211
M13 mp 19

FIG 6a

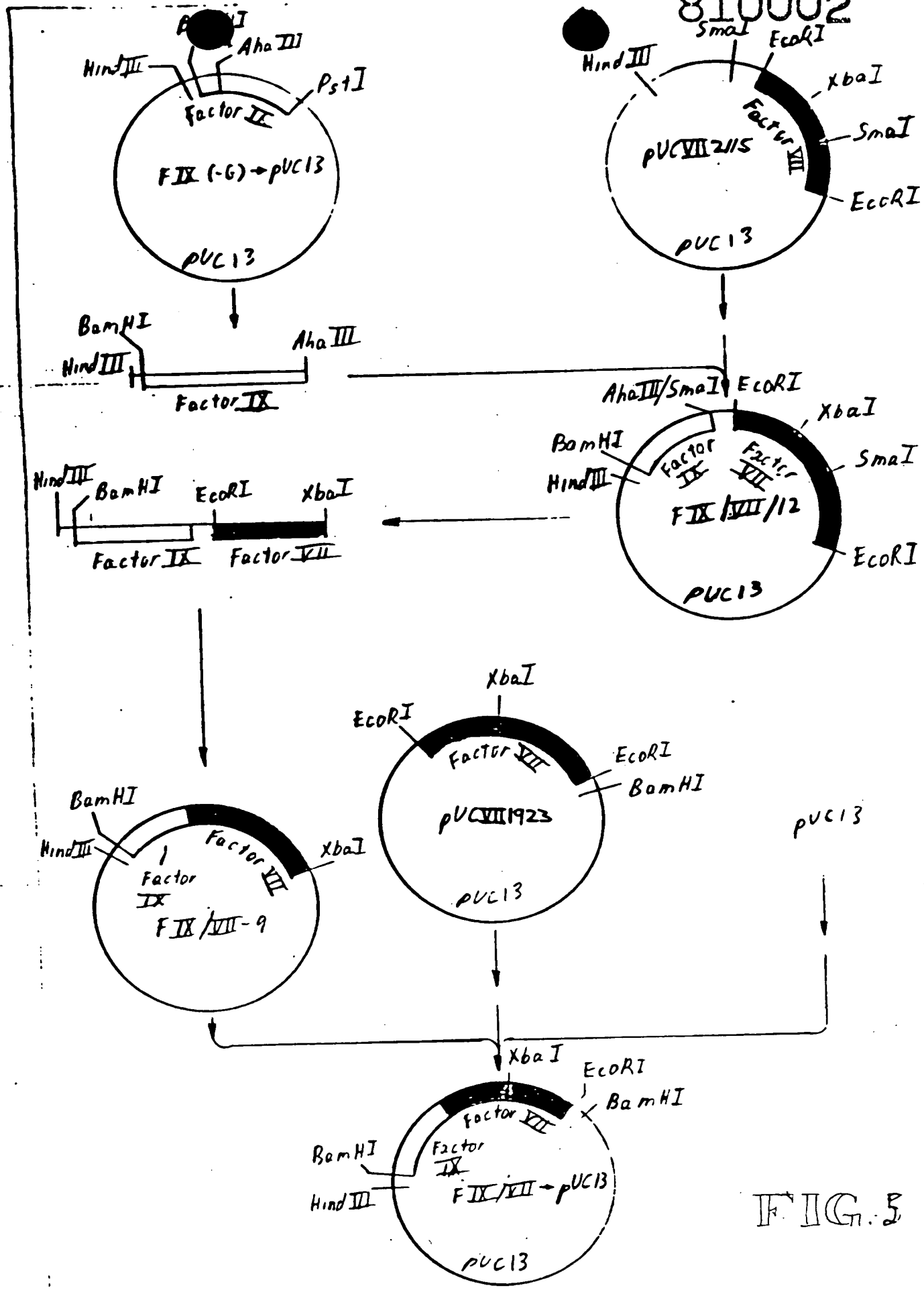

AhaIII
HindIII
PstI
Factor IX
FIX (-G)→pUC13
pUC13
SmaI
EcoRI
HindIII
XbaI
Factor VII
pUCVII2115
SmaI
EcoRI
pUC13
BamHI
HindIII
AhaIII
Factor IX
AhaIII/SmaI
EcoRI
XbaI
BamHI
Factor IX
Factor VII
HindIII
SmaI
FIX/VII/12
EcoRI
pUC13
HindIII
BamHI
EcoRI
XbaI
Factor IX
Factor VII
XbaI
EcoRI
Factor VII
EcoRI
BamHI
pUCVII1923
pUC13
pUC13
BamHI
HindIII
Factor VII
XbaI
Factor IX
FIX/VII-9
XbaI
EcoRI
BamHI
Factor VII
BamHI
Factor IX
HindIII
FIX/VII→pUC13
pUC13

FIG. 5

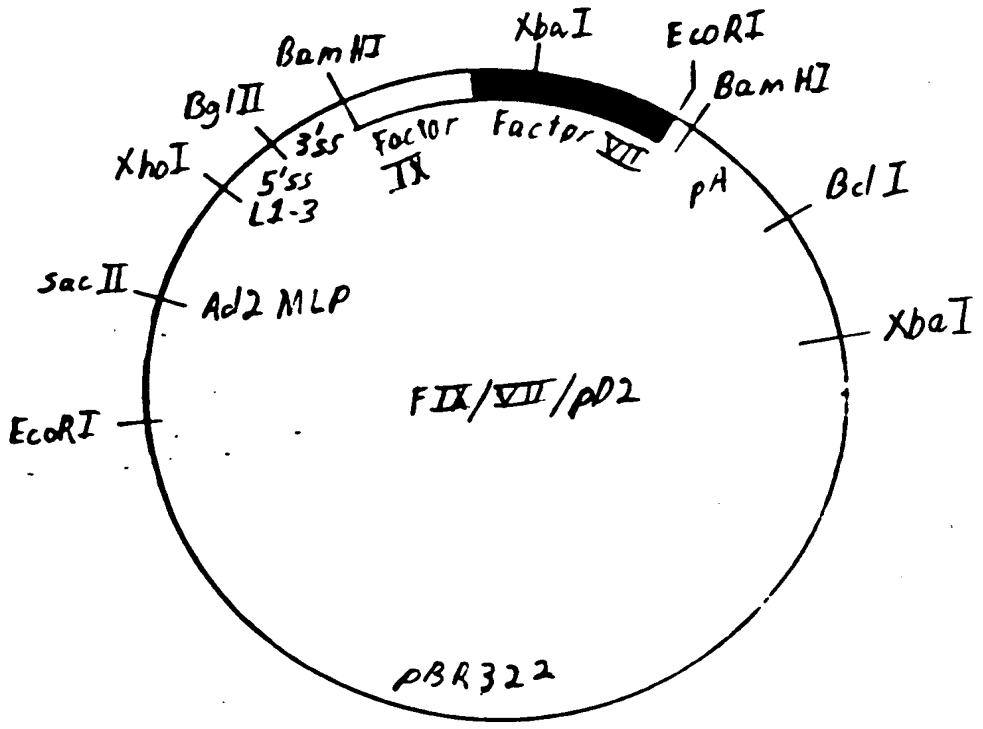

BamHI
XbaI
EcoRI
BamHI
BglII
3'ss
Factor IX
Factor VIII
XhoI
5'ss
L1-3
pA
BclI
SacII
Ad2 MLP
XbaI
FIX/VIII/pD2
EcoRI
pBR322

FIG. 6

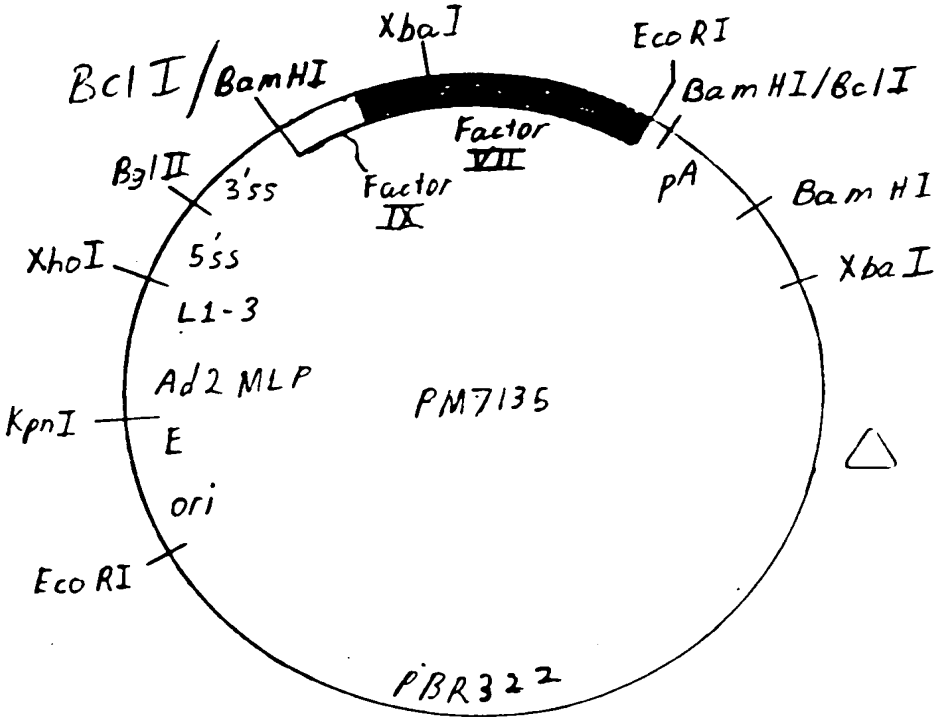

XbaI
EcoRI
BclI/BamHI
BamHI/BclI
Factor VIII
BglII
3'ss
Factor IX
pA
BamHI
XhoI
5'ss
XbaI
L1-3
Ad2 MLP
PM7135
KpnI
E
ori
EcoRI
pBR322

FIG. 8

FIGURE 7

```
                                21                                  36
GGATCC ATG CAG CGC GTG AAC ATG ATC ATG GCA GAA TCA CCA GGC
       MET Gln Arg Val Asn MET Ile MET Ala Glu Ser Pro Gly

                                66                                  81
CTC ATC ACC ATC TGC CTT TTA GGA TAT CTA CTC AGT GCT GAA TGT
Leu Ile Thr Ile Cys Leu Leu Gly Tyr Leu Leu Ser Ala Glu Cys

    96                          111                                 126
ACA GTT TTT CTT GAT CAT GAA AAC GCC AAC AAA ATT CTG AAT CGG
Thr Val Phe Leu Asp His Glu Asn Ala Asn Lys Ile Leu Asn Arg

   141                          156                                 171
CCA AAG AGG TAT AAT TCA GGT AAA TTG GAA GAG TTT GTT CAA GGG
Pro Lys Arg Tyr Asn Ser Gly Lys Leu Glu Glu Phe Val Gln Gly

   186                          201                                 216
AAC CTT GAG AGA GAA TGT ATG GAA GAA AAG TGT AGT TTT GAA GAA
Asn Leu Glu Arg Glu Cys MET Glu Glu Lys Cys Ser Phe Glu Glu

   231                          246                                 261
GCA CGA GAA GTT TTT GAA AAC ACT GAA AGA ACA AAG CTG TTC TGG
Ala Arg Glu Val Phe Glu Asn Thr Glu Arg Thr Lys Leu Phe Trp

   276                          291                                 306
ATT TCT TAC AGT GAT GGG GAC CAG TGT GCC TCA AGT CCA TGC CAG
Ile Ser Tyr Ser Asp Gly Asp Gln Cys Ala Ser Ser Pro Cys Gln

   321                          336                                 351
AAT GGG GGC TCC TGC AAG GAC CAG CTC CAG TCC TAT ATC TGC TTC
Asn Gly Gly Ser Cys Lys Asp Gln Leu Gln Ser Tyr Ile Cys Phe

   366                          381                                 396
TGC CTC CCT GCC TTC GAG GGC CGG AAC TGT GAG ACG CAC AAG GAT
Cys Leu Pro Ala Phe Glu Gly Arg Asn Cys Glu Thr His Lys Asp

   411                          426                                 441
GAC CAG CTG ATC TGT GTG AAC GAG AAC GGC GGC TGT GAG CAG TAC
Asp Glu Leu Ile Cys Val Asn Glu Asn Gly Gly Cys Glu Gln Tyr

   456                          471                                 486
TGC AGT GAC CAC ACG GGC ACC AAG CGC TCC TGT CGG TGC CAC GAG
Cys Ser Asp His Thr Gly Thr Lys Arg Ser Cys Arg Cys His Glu

   501                          516                                 531
GGG TAC TCT CTG CTG GCA GAC GGG GTG TCC TGC ACA CCC ACA GTT
Gly Tyr Ser Leu Leu Ala Asp Gly Val Ser Cys Thr Pro Thr Val

   546                          561                                 576
GAA TAT CCA TCT GGA AAA ATA CCT ATT CTA GAA AAA AGA AAT GCC
Glu Tyr Pro Cys Gly Lys Ile Pro Ile Leu Glu Lys Arg Asn Ala

   591                          606                                 621
AGC AAA CCC CAA GGC CGA ATT GTG GGG GGC AAG GTG TGC CCC AAA
Ser Lys Pro Gln Gly Arg Ile Val Gly Gly Lys Val Cys Pro Lys
```

```
   636                 651                 666
GGG GAG TGT CCA TGG CAG GTC CTG TTG TTG GTG AAT GGA GCT CAG
Gly Glu Cys Pro Trp Gln Val Leu Leu Leu Val Asn Gly Ala Gln

   681                 696                 711
TTG TGT GGG GGG ACC CTG ATC AAC ACC ATC TGG GTG GTC TCC GCG
Leu Cys Gly Gly Thr Leu Ile Asn Thr Ile Trp Val Val Ser Ala

   726                 741                 756
GCC CAC TGT TTC GAC AAA ATC AAG AAC TGG AGG AAC CTG ATC GCG
Ala His Cys Phe Asp Lys Ile Lys Asn Trp Arg Asn Leu Ile Ala

   771                 786                 801
GTG CTG GGC GAG CAC GAC CTC AGC GAG CAC GAC GGG GAT GAG CAG
Val Leu Gly Glu His Asp Leu Ser Glu His Asp Gly Asp Glu Gln

   816                 831                 846
AGC CGG CGG GTG GCG CAG GTC ATC ATC CCC AGC ACG TAC GTC CCG
Ser Arg Arg Val Ala Gln Val Ile Ile Pro Ser Thr Tyr Val Pro

   861                 876                 891
GGC ACC ACC AAC CAC GAC ATC GCG CTG CTC CGC CTG CAC CAG CCC
Gly Thr Thr Asn His Asp Ile Ala Leu Leu Arg Leu His Gln Pro

   906                 921                 936
GTG GTC CTC ACT GAC CAT GTG GTG CCC CTC TGC CTG CCC GAA CGG
Val Val Leu Thr Asp His Val Val Pro Leu Cys Leu Pro Glu Arg

   951                 966                 981
ACG TTC TCT GAG AGG ACG CTG GCC TTC GTG CGC TTC TCA TTG GTC
Thr Phe Ser Glu Arg Thr Leu Ala Phe Val Arg Phe Ser Leu Val

   996                1011                1026
AGC GGC TGG GGC CAG CTG CTG GAC CGT GGC GCC ACG GCC CTG GAG
Ser Gly Trp Gly Gln Leu Leu Asp Arg Gly Ala Thr Ala Leu Glu

  1041                1056                1071
CTC ATG GTC CTC AAC GTG CCC CGG CTG ATG ACC CAG GAC TGC CTG
Leu MET Val Leu Asn Val Pro Arg Leu MET Thr Gln Asp Cys Leu

  1086                1101                1116
CAG CAG TCA CGG AAG GTG GGA GAC TCC CCA AAT ATC ACG GAG TAC
Gln Gln Ser Arg Lys Val Gly Asp Ser Pro Asn Ile Thr Glu Tyr

  1131                1146                1161
ATG TTC TGT GCC GGC TAC TCG GAT GGC AGC AAG GAC TCC TGC AAG
MET Phe Cys Ala Gly Tyr Ser Asp Gly Ser Lys Asp Ser Cys Lys

  1176                1191                1206
GGG GAC AGT GGA GGC CCA CAT GCC ACC CAC TAC CGG GGC ACG TGG
Gly Asp Ser Gly Gly Pro His Ala Thr His Tyr Arg Gly Thr Trp

  1221                1236                1251
TAC CTG ACG GGC ATC GTC AGC TGG GGC CAG GGC TGC GCA ACC GTG
Tyr Leu Thr gly Ile Val Ser Trp Gly Gln Gly Cys Ala Thr Val
```

```
  1266                1281                1296
GGC CAC TTT GGG GTG TAC ACC AGG GTC TCC CAG TAC ATC GAG TGG
Gly His Phe Gly Val Tyr Thr Arg Val Ser Gln Tyr Ile Glu Trp

  1311                1326                1341
CTG CAA AAG CTC ATG CGC TCA GAG CCA CGC CCA GGA GTC CTC CTG
Leu Gln Lys Leu MET Arg Ser Glu Pro Arg Pro Gly Val Leu Leu

  1356                          1378      1388      1398
CGA GCC CCA TTT CCC TAG CCCAGCAGCC CTGGCCTGTG GAGAGAAAGC
Arg Ala Pro Phe Pro  .

      1408      1418      1428      1438      1448
CAAGGCTGCG TCGAACTGTC CTGGCACCAA ATCCCATATA TTCTTCTGCA

      1458      1468      1478      1488      1498
GTTAATGGGG TAGAGGAGGG CATGGGAGGG AGGGAGAGGT GGGGAGGGAG

      1508      1518      1528      1538      1548
ACAGAGACAG AAACAGAGAG AGACAGAGAC AGAGAGAGAC TGAGGGAGAG

      1558      1568      1578      1588      1598
ACTCTGAGGA CCATGGAGAG AGACTCAAAG AGACTCCAAG ATTCAAAGAG

      1608      1618      1628      1638      1648
ACTAATAGAG ACACAGAGAT GGAATAGAAA AGATGAGAGG CAGAGGCAGA

      1658      1668      1678      1688      1698
CAGGCGCTGG ACAGAGGGGC AGGGGAGTGC CAAGGTTGTC CTGGAGGCAG

      1708      1718      1728      1738      1748
ACAGCCCAGC TGAGCCTCCT TACCTCCCTT CAGCCAAGCC CCACCTGCAC

      1758      1768      1778      1788      1798
GTGATCTGCT GGCCCTCAGG CTGCTGCTCT GCCTTCATTG CTGGAGACAG

      1808      1818      1828      1838      1848
TAGAGGCATG ACACACATGG ATGCACACAC ACACACGCCA TGCACACACA

      1858      1868      1878      1888      1898
CAGAGATATG CACACACACG GATGCACACA CAGATGGTCA CACAGAGTAC

      1908      1918      1928      1938      1948
GCAAACACAC CGATGCACAC GCACATAGAG ATATGCACAC ACAGATGCAC
```

```
      1958        1968        1978        1988        1998
ACACAGATAT ACACATGGAG TGCACGCACA TGCCAATGCA CGCACACATC

      2008        2018        2028        2038        2048
AGTGCACACG GATGCACAGA GATATGCACA CACCGATGTG CGCACACACA

      2058        2068        2078        2088        2098
GATATGCACA CACATGGATG AGCACACACA CACCAAGTGC GCACACACAC

      2108        2118        2128        2138        2148
CGATGTACAC ACAGATGCAC ACACAGATGC ACACACACCG ATGCTGACTC

      2158        2168        2178        2188        2198
CATGTGTGCT GTCCTCTGAA GGCGGTTGTT TAGCTCTCAC TTTTCTGGTT

      2208        2218        2228        2238        2248
CTTATCCATT ATCATCTTCA CTTCAGACAA TTCAGAAGCA TCACCATGCA

      2258        2268        2278        2288        2298
TGGTGGCGAA TGCCCCCAAA CTCTCCCCCA AATGTATTTC TCCCTTCGCT

      2308        2318        2328        2338        2348
GGGTGCCGGG CTGCACAGAC TATTCCCCAC CTGCTTCCCA GCTTCACAAT

      2358        2368        2378        2388        2398
AAACGGCTGC GTCTCCTCGC AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA

      2408        2418        2428        2438
AAAAAAAAAA AAGGAATTCG AGCTCGGTAC CCGGGGATCC
```

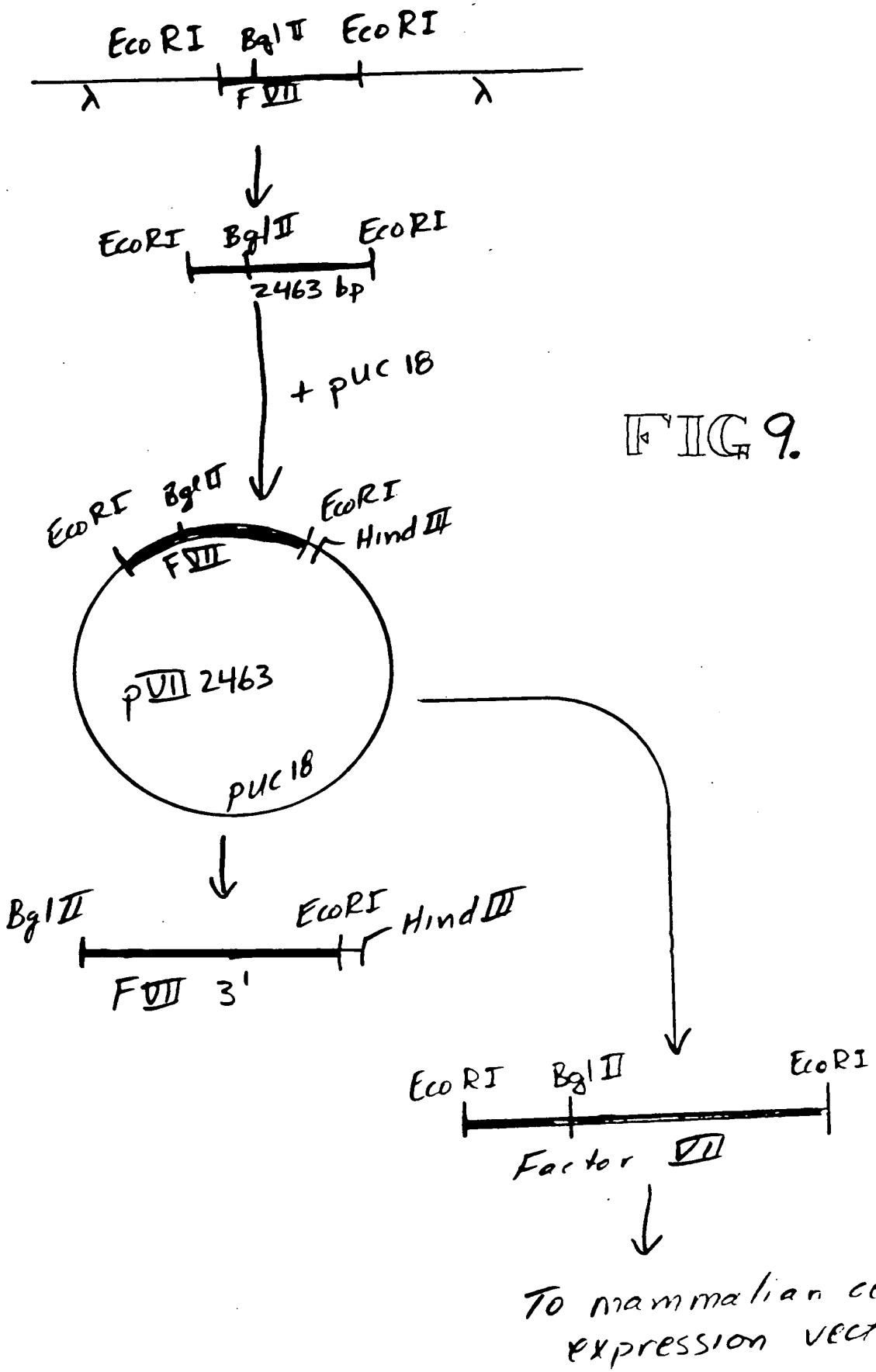

EcoRI BglII EcoRI
λ
FVII
λ
EcoRI BglII EcoRI
2463 bp
+ pUC 18
EcoRI BglII
EcoRI
Hind III
FVII
pVII 2463
pUC 18
BglII
EcoRI
Hind III
FVII 3'
EcoRI BglII EcoRI
Factor VII
To mammalian cell expression vector

FIG 9.

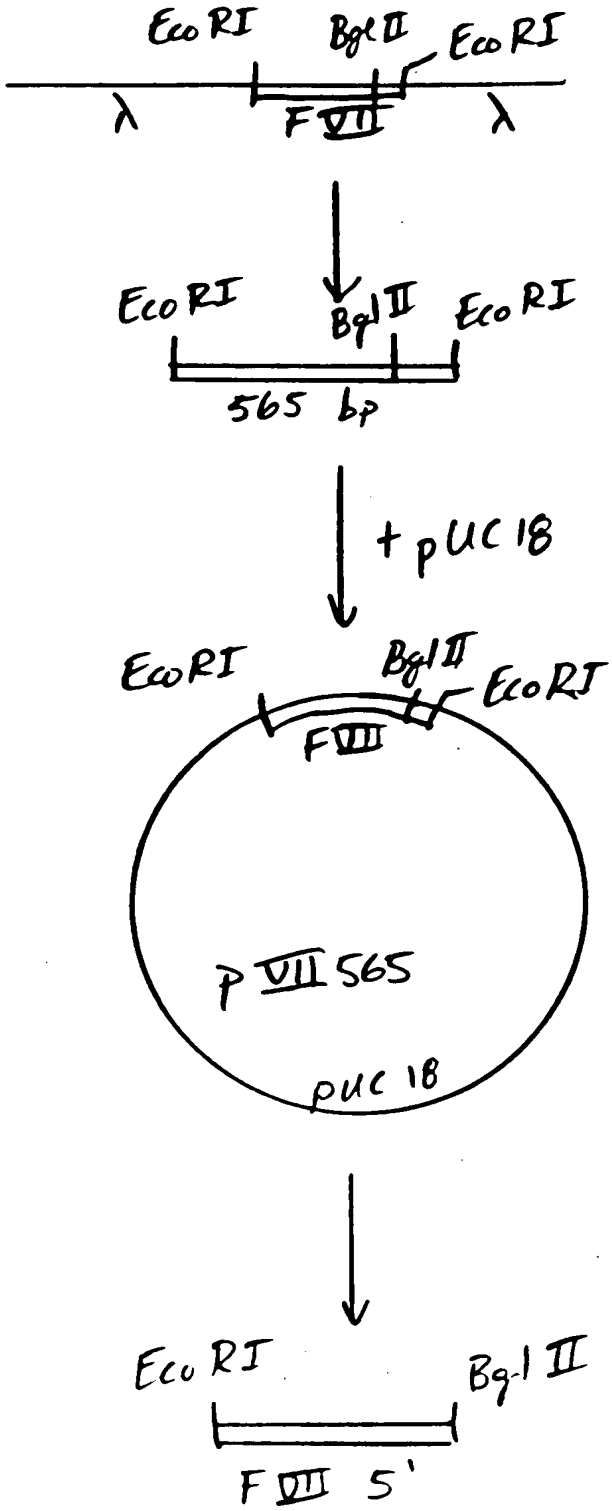

EcoRI
BglII
EcoRI
λ
FVII
λ
EcoRI
BglII
EcoRI
565 bp
+pUC18
EcoRI
BglII
EcoRI
FVII
pVII565
puc18
EcoRI
Bg-l II
FVII 5'

FIG 10.

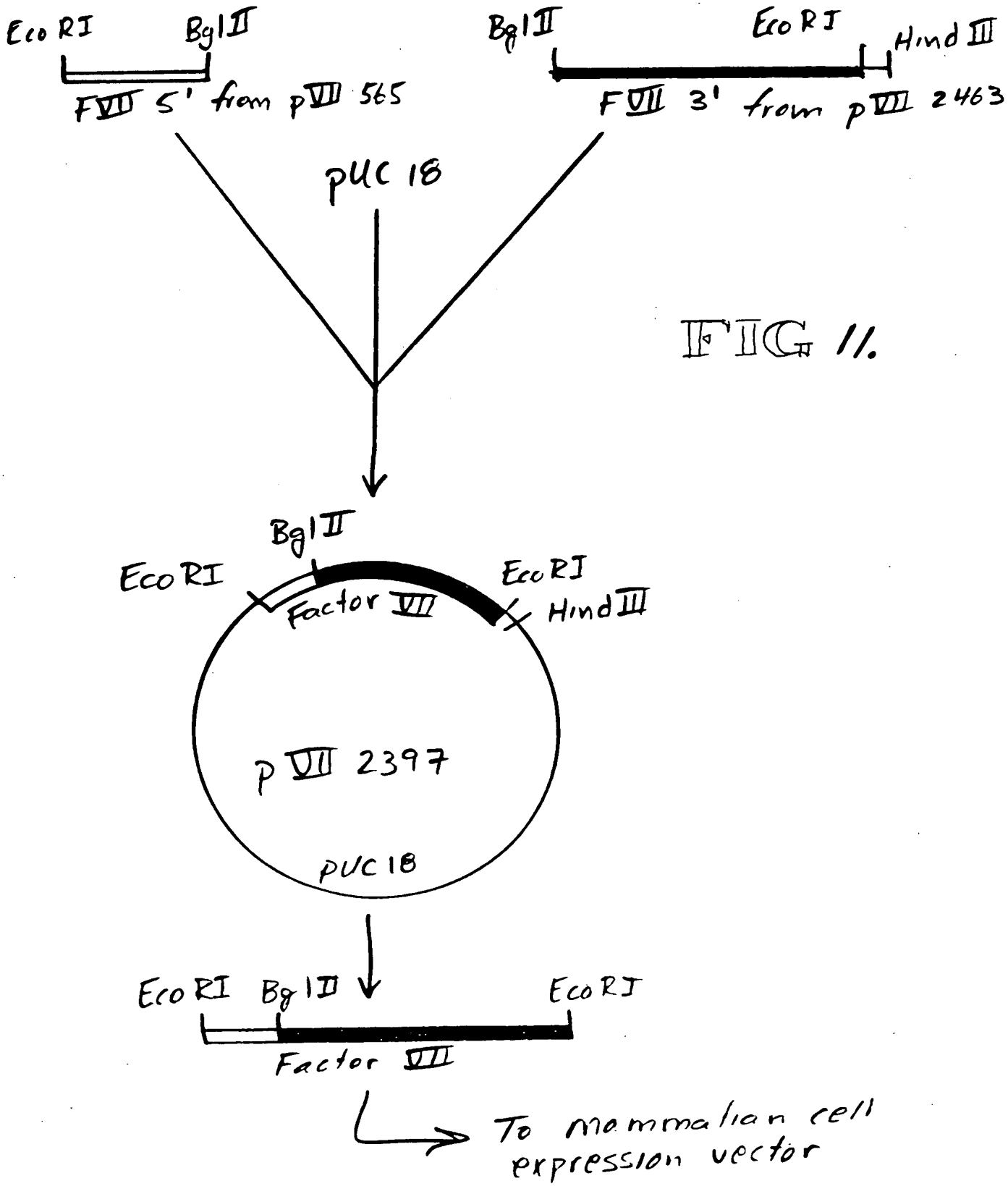
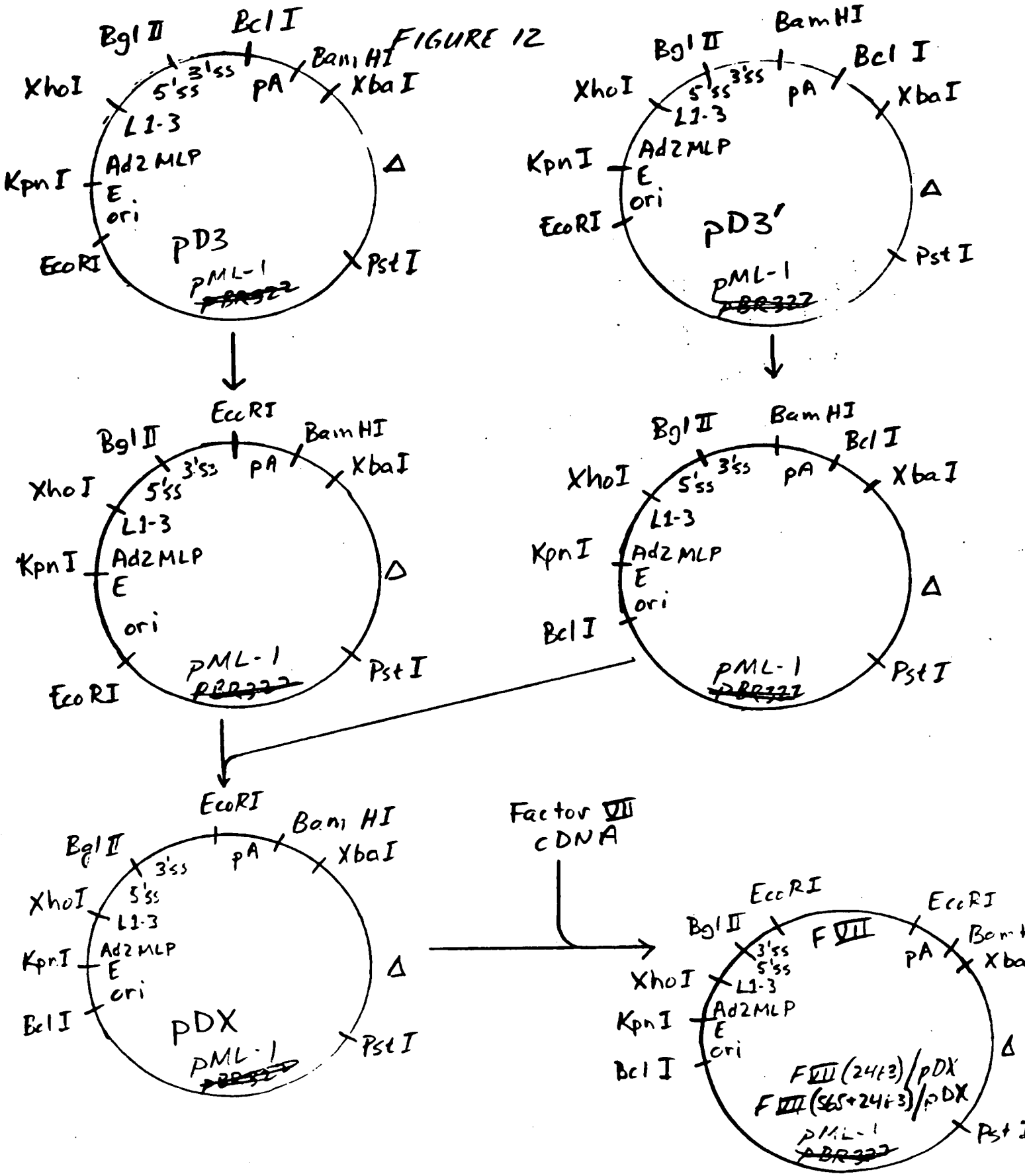

Eco RI
Bgl II
FVII 5' from pVII 565
Bgl II
Eco RI
Hind III
FVII 3' from pVII 2463
pUC 18
Bgl II
Eco RI
Eco RI
Factor VII
Hind III
pVII 2397
pUC 18
Eco RI
Bgl II
Eco RI
Factor VII
To mammalian cell expression vector

FIG 11.

FIGURE 12


Bgl II
Bcl I
Bam HI
XhoI
5'ss
3'ss
pA
XbaI
L1-3
Ad2 MLP
KpnI
E
ori
Δ
pD3
EcoRI
PstI
pML-1
pBR322
pD3'
EcoRI
BclI
pDX
Factor VIII
cDNA
F VIII (24+3)/pDX
F VIII (565+24+3)/pDX

```
                714                   729                   744
CGG ACG TTC TCT GAG AGG ACG CTG GCC TTC GTG CGC TTC TCA TTG GTC AGC GGC
Arg Thr Phe Ser Glu Arg Thr Leu Ala Phe Val Arg Phe Ser Leu Val Ser Gly

759                   774      Nar I        789                   804
TGG GGC CAG CTG CTG GAC CGT GGC GCC ACG GCC CTG GAG CTC ATG GTC CTC AAC
Trp Gly Gln Leu Leu Asp Arg Gly Ala Thr Ala Leu Glu Leu Met Val Leu Asn

        819                   834      Pst Ib       849                   864
GTG CCC CGG CTG ATG ACC CAG GAC TGC CTG CAG CAG TCA CGG AAG GTG GGA GAC
Val Pro Arg Leu Met Thr Gln Asp Cys Leu Gln Gln Ser Arg Lys Val Gly Asp

                879                   894                   909
TCC CCA AAT ATC ACG GAG TAC ATG TTC TGT GCC GGC TAC TCG GAT GGC AGC AAG
Ser Pro Asn Ile Thr Glu Tyr Met Phe Cys Ala Gly Tyr Ser Asp Gly Ser Lys

    924                   939                   954                   969
GAC TCC TGC AAG GGG GAC AGT GGA GGC CCA CAT GCC ACC CAC TAC CGG GGC ACG
Asp Ser Cys Lys Gly Asp Ser Gly Gly Pro His Ala Thr His Tyr Arg Gly Thr

            984                   999                  1014
TGG TAC CTG ACG GGC ATC GTC AGC TGG GGC CAG GGC TGC GCA ACC GTG GGC CAC
Trp Tyr Leu Thr Gly Ile Val Ser Trp Gly Gln Gly Cys Ala Thr Val Gly His

1029                  1044                  1059 TaqI             1074
TTT GGG GTG TAC ACC AGG GTC TCC CAG TAC ATC GAG TGG CTG CAA AAG CTC ATG
Phe Gly Val Tyr Thr Arg Val Ser Gln Tyr Ile Glu Trp Leu Gln Lys Leu Met

        1089                  1104                  1119                  1138
CGC TCA GAG CCA CGC CCA GGA GTC CTC CTG CGA GCC CCA TTT CCC TAG CCCAGCAGCC
Arg Ser Glu Pro Arg Pro Gly Val Leu Leu Arg Ala Pro Phe Pro
                                                                    PstIc
      1148       1158       1168       1178       1188       1198       1208
CTGGCCTGTG GAGAGAAAGC CAAGGCTGCG TCGAACTGTC CTGGCACCAA ATCCCATATA TTCTTCTGCA

      1218       1228       1238       1248       1258       1268       1278
GTTAATGGGG TAGAGGAGGG CATGGGAGGG AGGGAGAGGT GGGGAGGGAG ACAGAGACAG AAACAGAGAG

      1288       1298       1308       1318       1328       1338       1348
AGACAGAGAC AGAGAGAGAC TGAGGGAGAG ACTCTGAGGA CCATGGACAG AGACTCAAAG AGACTCCAAG

      1358       1368       1378       1388       1398       1408       1418
ATTCAAAGAG ACTAATAGAG ACACAGAGAT GGAATAGAAA AGATGAGAGG CAGAGGCAGA CAGGCGCTGG

      1428       1438       1448       1458       1468       1478       1488
ACAGAGGGGC AGGGGAGTGC CAAGGTTGTC CTGGAGGCAG ACAGCCCAGC TGAGCCTCCT TACCTCCCTT
```

```
      1498       1508       1518       1528       1538       1548       1558
CAGCCAAGCC CCACCTGCAC GTGATCTGCT GGCCCTCAGG CTGCTGCTCT GCCTTCATTG CTGGAGACAG

      1568       1578       1588       1598       1608       1618       1628
TAGAGGCATG ACACACATGG ATGCACACAC ACACACGCCA TGCACACACA CAGAGATATG CACACACACG

      1638       1648       1658       1668       1678       1688       1698
GATGCACACA CAGATGGTCA CACAGAGTAC GCAAACACAC CGATGCACAC GCACATAGAG ATATGCACAC

      1708       1718       1728       1738       1748       1758       1768
ACAGATGCAC ACACAGATAT ACACATGGAG TGCACGCACA TGCCAATGCA CGCACACATC AGTGCACACG

      1778       1788       1798       1808       1818       1828       1838
GATGCACAGA GATATGCACA CACCGATGTG CGCACACACA GATATGCACA CACATGGATG AGCACACACA

      1848       1858       1868       1878       1888       1898       1908
CACCAAGTGC GCACACACAC CGATGTACAC ACAGATGCAC ACACAGATGC ACACACACCG ATGCTGACTC

      1918       1928       1938       1948       1958       1968       1978
CATGTGTGCT GTCCTCTGAA GGCGGTTGTT TAGCTCTCAC TTTTCTGGTT CTTATCCATT ATCATCTTCA

      1988       1998       2008       2018       2028       2038       2048
CTTCAGACAA TTCAGAAGCA TCACCATGCA TGGTGGCGAA TGCCCCCAAA CTCTCCCCCA AATGTATTTC

      2058       2068       2078       2088       2098       2108       2118
TCCCTTCGCT GGGTGCCGGG CTGCACAGAC TATTCCCCAC CTGCTTCCCA GCTTCACAAT AAACGGCTGC

      2128       2138       2148       2158       2168     EcoRIb
GTCTCCTCGC AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAGGAATTC
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